

# PRELIMINARY STUDIES ON THE EXTRACTION OF PHENOLIC COMPOUNDS FROM OLIVE LEAVES OF PORTUGUESE CULTIVARS

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## Introduction

Phenolic compounds of olive tree (*Olea europaea*) are considered one of the responsables of the health-promoting of Mediterranean diet [1]. Indeed, recent studies on isolated phenolic compounds have found a number of applications in increasing human immune response [2]. The olive leaves, known to be rich in phenolic compounds, are a by-product of pruning and mechanical harvesting of olives. In the present work, started in November 2015, optimization in extraction and in component analysis has been achieved.

## Materials and Methods

Olive leaves of Galega Vulgar (G) and Cobrançosa (C) varieties with 8 years old (Fig. 1) were collected in November (N), March (M) and July (J). After drying at 50 °C and scrunching with an electric mill, extractions were performed with ethanol/water (3 successive extractions, 12 hours each at room temperature). After removing the solvent with a rotavapor, the yeld was calculated as % mass extracted per mass of dried leaf. Thin layer chromatography (TLC) of the ethanolic extracts was performed on a silica gel plate with toluene/ethyl acetate/acetic acid, 35/40/25; spots were detected by iodine vapor (Fig. 2)

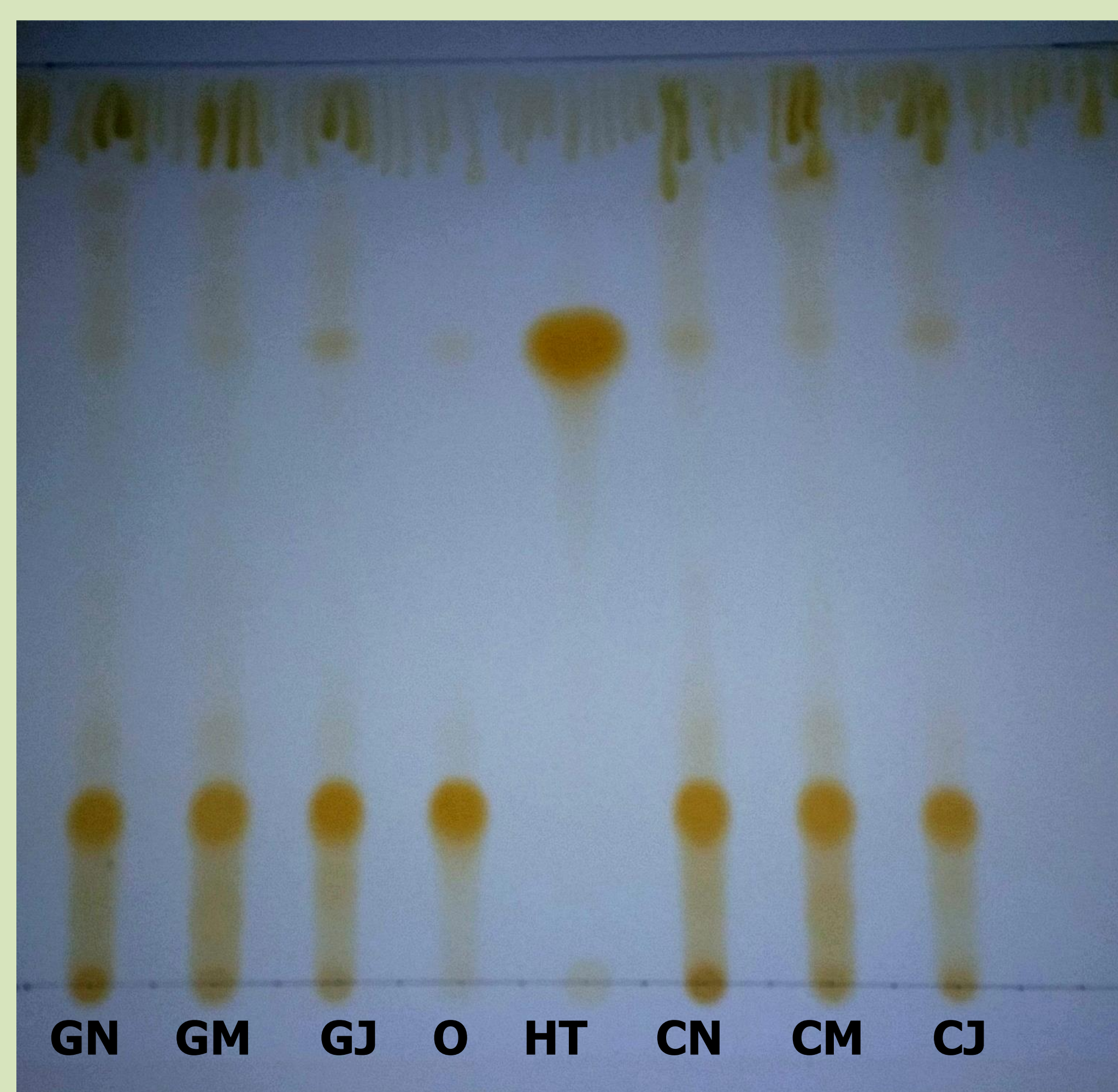
## Results & Discussion

The yield of ethanolic fraction of Galega and Cobrançosa were different ( $p < 0.05$ ) in November and July (Galega > Cobrançosa) but did not differ in March. The yields in November were lower than those of March and July, for both varieties. The results by TLC suggest that the general composition is not very different between samples, but these preliminary results need to be confirmed. It is interesting to note that although some hydroxytyrosol (HT) was detected, most of the oleuropein (O) remained intact (Fig. 2). This fact showed that the entire procedure used, from the collection, drying, storage, extraction, evaporation and re-storage, did not destroy the larger secoiridoid of the leaf. In other words, the optimized process allowed the obtainment of oleuropein. If desired, a subsequent hydrolysis, by chemical or enzymatic or microbiological methods, may be performed in order to produce derivatives of lower molecular weight such as hydroxytyrosol.

As far as we know, this TLC method for the simultaneous analysis of oleuropein and hydroxytyrosol, without derivatization, is not described in the literature.



**Fig.1** – Olive leaves of Galega (left) and Cobrançosa (right)



**Fig.2** - TLC of ethanolic extracts from Galega and Cobrançosa leaves, collected in November, March and July. Comparison with oleuropein (O) and hydroxytyrosol (HT) standards

## References

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- [2] Lockyer S, Rowland I, Spencer JP, Yaqoob P, Stonehouse W. Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomised controlled trial. *European journal of nutrition*. 2016;7:1-12.

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